

Inhibition by Rutin of Capillary Injury by Alpha-Radiation in Rats

By JOHN Q. GRIFFITH, JR., M.D. AND JAMES F. COUCH, PH.D.

A CONVENIENT LABORATORY METHOD for determining the activity of various substances in preserving or restoring the integrity of the capillary wall has long been desired. Methods that depend upon the production of cutaneous petechiae by either negative or positive pressure are open to certain objections, and results obtained by such technics are not generally accepted. A test of this kind is of special importance in investigating the so-called vitamin P action of the flavonoids. Other technics have been employed: the "sparing" action on epinephrin,¹⁻³ permeability of capillaries to dyes⁴ and prevention of irradiation injury.⁵⁻⁸

None of these has been universally accepted as definitely indicating a vitamin P action and some have been severely criticized.⁹

Recently the technic of Griffith, Anthony, Pendergrass and Perryman¹⁰ has been adapted to the determination of the protective action of rutin on the peritoneal capillaries. Radon ointment, a source of alpha radiation, is introduced into the peritoneal cavity of normal rats in quantities sufficient to provide 40 to 200 μ c. This ointment contains small quantities of radon gas in either lanolin or olive oil.* Alpha particles comprise 90 per cent of the radiation; the remainder consists of beta particles and gamma rays. After ten days, increased capillary fragility may be demonstrated in the peritoneal circulation by the following procedure:

Under general anesthesia the peritoneal cavity is opened by a long mid-line longitudinal incision, care being taken to exert no traction on the peritoneum. The abdominal wall is carefully folded back until an artery and vein are disclosed. Then, under a good light, the peritoneum is grasped on each side of the vessels and stretched between the fingers so that the lumen of the vein is obliterated but the artery can still be seen pulsating. The tension is maintained for two minutes, after which it is relaxed. The peritoneal area supplied by the vessels is carefully inspected and, if the fragility is increased, one or more petechiae will appear distal to the point of venous obstruction, usually entirely separate from any visible vessel. In a positive case, six or eight such hemorrhages usually occur. If desired, the process can be repeated in the same animal, using other vessels. No petechial hemorrhages occur in the normal animal.

When rutin was given, it was always given for the first time ten to thirty

minutes following the introduction of the radon ointment. It was never given for the first time later, after increase in capillary fragility had been demonstrated.

Animals protected by rutin behaved like normal rats as indicated in table 1. Rutin in pellets was inserted subcutaneously every third day, or a solution

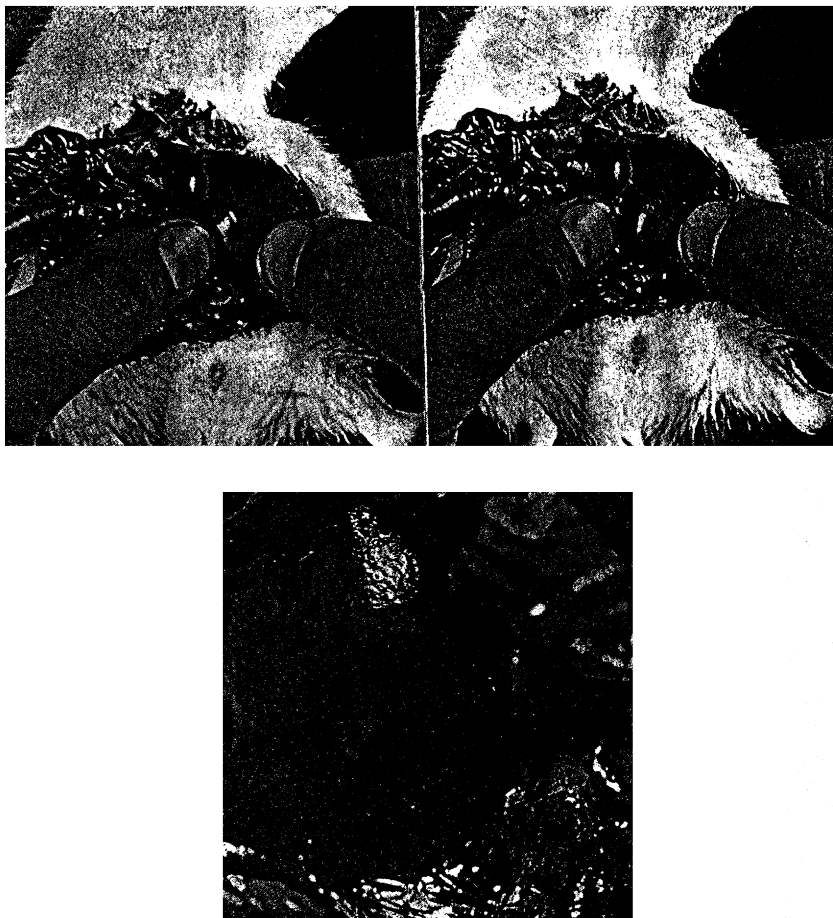


FIG. 1.—The upper views show position of the hands in constricting a peritoneal vein. On the left, the peritoneum is stretched and the vein appears interrupted for a short segment, while the artery is unaffected. On the right, the hands are in position but no tension is applied so that both artery and vein show throughout. Below, the hands have been removed after tension is released, and multiple petechiae are seen. The white areas are due to light reflex.

(rutin-methyl glucamine complex¹²) was administered subcutaneously daily. Five mg. of rutin by pellet afforded no protection against 40 μ c of radiation, but 20 mg. by pellet protected 18 of 21 rats against 200 μ c, 10 out of 10 against 80 μ c, and 11 out of 11 against 40 μ c. In solution, 12.5 mg. of rutin gave good pro-

tection (11 out of 12) against 80 μ c and 25 mg. gave complete protection (6 out of 6). Ten mg. of ascorbic acid daily for ten days gave no significant protection. Unprotected rats given 200 μ c of radiation showed no increased capillary fragility at the end of ninety days, indicating an autogenous ability to correct the condition with the exhaustion of the exciting cause. All animals remained healthy during the experiments, and there were no spontaneous deaths.

TABLE 1.—*Protection by Rutin of Capillary Integrity against Alpha-Radiation*

No. of rats	Duration of test	Dose of radiation	Dose of rutin		Ascorbic acid daily	Capillary fragility	
			pellet	third day		Normal	Abnormal
			mg.	mg.		no.	no.
11	—	none	none	none	none	11	0
30	12-25	200	none	none	none	5	25
21	12-25	200	20		none	18	3
10	.10	40	none	none	none	5	5
11	10	40	5		none	6	5
11	10	40	20		none	11	0
19	10	80	none	none	none	8	11
10	10	80	20		none	10	0
12	10	80		12.5	none	11	1
6	10	80		25	none	6	0
6	10	80	none	none	10	3	3
6	90	200	none	none	none	6	0

SUMMARY

Rutin administered subcutaneously or by implantation can protect the rat against increased capillary fragility due to irradiation with alpha particles.

REFERENCES

- ¹ JAVILLIER, M. AND LAVOLLAY, J.: Les substances sur la résistance et la perméabilité capillaires et la notion de vitamine P. *Helvet. chim. acta.* 29: 1283-1289, 1946.
- ² LAVOLLAY, J.: La vitamine P. *Compt. rend. Soc. de biol.* 137: 23-24, 1943.
- ³ WILSON, ROBERT H., MORTAROTTI, T. G. AND DEEDS, FLOYD: Some pharmacological properties of rutin. *J. Pharmacol. & Exper. Therap.* 90: 120-127, 1947.
- ⁴ AMBROSE, ANTHONY M. AND DEEDS, FLOYD: Effect of rutin on permeability of cutaneous capillaries. *J. Pharmacol. & Exper. Therap.* 90: 359-363, 1947.
- ⁵ GRIFFITH, J. Q., JR., ANTHONY, ELEANOR, PENDERGRASS, E. P. AND PERRYMAN, R.: Effect of rutin on recovery time from radiation injury in rats. *Proc. Exper. Biol. & Med.* 64: 332-333, 1947.
- ⁶ CLARK, W. G., UNCAPHER, R. P. AND JORDAN, M. L.: Effect of flavonoids (vitamin P) on mortality from total body roentgen irradiation. *Science* 108: 629-630, 1948.
- ⁷ SOKOLOFF, B. T., REDD, J. B. AND DUTCHER, R.: *Science* 112: 112-113, 1950.
- ⁸ REKERS, PAUL E. AND FIELD, JOHN B.: Control of hemorrhagic syndrome and reduction in x-irradiation mortality with a flavonone. *Science* 107: 16-17, 1948.
- ⁹ SOKOLOFF, B. T. AND REDD, J. B.: Study on vitamin P. Florida Southern College, Monograph I, 1950.
- ¹⁰ GRIFFITH, J. Q., JR., ANTHONY, ELEANOR, PENDERGRASS, E. P. AND PERRYMAN, R.: Production of increased capillary fragility in rats following irradiation. *Proc. Soc. Exper. Biol. & Med.* 64: 331-332, 1947.
- ¹¹ PLUNGIAN, M. B.: U. S. Patent No. 2,451,772, October 19, 1948.